Importance of soil toxicity in relation to the stability of plant communities

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Introduction

It is unusual to find in a mesophytic environment any one factor able to dictate completely the pattern of plant associations, and in this respect soil toxicity is no exception. Nevertheless, in some communities there is little doubt that toxic compounds in the soil do exert a marked differential effect on the growth of competing higher plants. The origin of these compounds is usually a higher plant, either as a growing entity or as a source of material for decomposition, and the toxins produced may be either fungitoxic or phytotoxic in action. Many species have been reported as providing sources of metabolites which are toxic to other plants (Bonner 1950; Woods 1960), and for this reason the material in this paper refers only to situations where the stability and species composition of an entire community is, at least in part, determined by toxic factors in the soil. In particular the influence of such factors in relation to the dominance of *Calluna vulgaris* (Hull) over certain heathland areas is considered.

Evidence suggesting the presence of toxic compounds in heathland soils

It has long been known that heathlands are inhospitable sites for afforestation, and Muller (1903) was perhaps the first to observe the 'checking' of growth of Norway spruce and silver fir on *Calluna* heathlands in western Jutland. Satisfactory growth of the spruce took place only for as long as re-invasion of the sites by *Calluna* did not occur. It has been suggested that competition for available nutrients or soil moisture are responsible for this 'checking' of spruce by *Calluna*, but Braathe (1950) sees these factors as subsidiary to a biological effect on spruce by *Calluna* itself. Changes in the root systems of

'checked' Sitka spruce following the killing of *Calluna* around their bases have been described by Yeatman (1955). Where the *Calluna* was killed by mulching, the first reaction of the spruce was to produce a crop of adventitious roots between the mulch and the original peat. Then, over the next three years, the original root system formed in the nursery began to develop and a normal overall growth rate for spruce was resumed. The same author was of the opinion that an increased availability of nutrients was not responsible for the resumed growth. The obvious conclusion remains, therefore, that the inhibition of root extension was due to some antagonistic factor in the *Calluna* peat, a view which has been confirmed in this laboratory.

The idea that the superficial organic layer of heathland soils contains substances inimical to root growth and the formation of ectotrophic mycorrhizas had already been suggested by Rayner & Neilson-Jones (1944). However, in the absence of concrete evidence as to the nature of this inhibitory material or its origin their conclusions met with some scepticism. Brian, Hemming & McGowan (1945) carried the investigation a stage further by suggesting that antibiotics from the normal saprophytic microflora might be implicated, at least in suppressing the formation of mycorrhizas. However, further study soon established that antibiotics are unlikely to occur in significant amounts in the soil. In this present investigation the toxicity of *Calluna* peat to the growth of certain plant species was assumed to be due to two discrete types of toxic metabolite; one, phytotoxic in nature and derived from decaying litter, the other, fungitoxic and having its origin in the living *Calluna* plant. It was further postulated that both could be equally effective in relation to the stability and species composition of *Calluna* heathlands.

Phytotoxic activity of *Calluna* raw humus

The work of Yeatman, already mentioned, and that of Laing (1932) indicates an almost total absence of root development by spruce in the organic layer of heathland soils. The same is true where the colonization of heathland has been attempted by seedlings of indigenous species, for example, birch. Available field evidence indicates that this condition could be due to some phytotoxic component within *Calluna* raw humus. This conclusion was supported by a factorial experiment designed to determine the extent to which factors, such as acid pH, low availability of nutrients or unsuitable soil moisture content, influenced the growth of wheat (var Koga II) in pots containing a mixture of non-calcareous grit and *Calluna* peat. The results of this experiment and other preliminary trials with different test species, e.g. peas, and tomatoes, clearly showed that some material from *Calluna* raw humus was capable of preventing root development, irrespective of how other factors were manipulated.

To be ecologically active it is probable that a factor of the type proposed would have to be water-soluble, and the validity of this surmise was tested by growing seedlings in sand culture; the nutrient solution of Hoagland & Arnon (1938) was used amended by water extracts of *Calluna* peat. Seedlings of horticultural crops were employed simply to facilitate experimentation but, where possible, specific aspects of the basic hypothesis were examined further using species which might be expected to compete with *Calluna* under field conditions. To obtain an aqueous extract containing the toxic factor, raw humus (500 g) was fragmented into a 2 litre flask together with 500 ml of distilled water. It was then left to stand for 12 hours. After this time the liquid was removed from the organic matter by means of a press, followed by filtration through two layers of muslin. The product was a brown, opalescent fluid, and one such preparation was used to amend the nutrient solutions for the sand culture experiment referred to above; in each case (see Table I) the final concentration of nutrients was the same.

The roots of the test plants showed a restriction of growth correlated with increasing concentration of peat extract, but in no case could any sign of fungal invasion be discerned. It is clear, therefore, that *Calluna* peat does contain

Table 1. Effect of water extract of *Calluna* peat on root growth of the test seedlings indicated.

Test material	Mean root length in cm (average of 20 plants sampled at 14 days)				
	% extract present in 35 ml of solution				
	0	25	50	75	100
Wheat	7·5	5·4	1·5	0·6	0·5
Barley	8·3	4·8	2·2	0·4	0·5
Pea	5·2	2·4	1·2	0·2	0·0
Sweet pea	6·1	2·8	0·9	0·3	0·0
Tomato	4·8	3·1	1·2	0·8	0·4

a water-soluble factor capable of inhibiting the growth of roots of certain higher plants.

It was further possible to inhibit the growth of isolated pea roots with water-extracts of *Calluna* peat under aseptic conditions employing the technique of Torrey (1959), and to correlate this restricted growth with an inhibition of oxygen uptake by similar root material. Comparable results were obtained employing seedlings of tomato. The diminished oxygen uptake induced by these water-extracts formed a useful assay for the presence of the phytotoxic factor, and the following procedure was adopted as a standard means of screening samples of *Calluna* raw humus for phytotoxicity.

Tomato seeds (Moneymaker) were surface sterilized with hypochlorite solution and incubated on moist filter paper (sterile) in the dark for 4 days. Batches of 50 germinated seeds with radicles approximately 5–10 mm in length were selected and placed in 100 ml of a water-extract of *Calluna* peat for 12 hours. After this time the seedlings were washed in distilled water and transferred to 3·0 ml of phosphate buffer (M/15 Na_2HPO_4/KH_2PO_4, pH 4·5) in Warburg flasks; carbon dioxide was absorbed by 20 per cent KOH. Four flasks were used with each test treatment, and each experiment was repeated at least three times. The control treatment was similar except that the soil extract was prepared from a grassland loam soil (pH 4·2); the influence of peat extracts on oxygen uptake by the test roots was referred to this standard.

This procedure gave a simple and reproducible means of detection of the phytotoxin, and Fig. 1 shows the results of a typical series of assays carried out during August 1968. Similar results were obtained using roots from both wheat (var. Koga) and peas (var. Onward) and with roots of *Picea abies*. Although one cannot, on the basis of laboratory results, directly suggest an ecological significance for an occurrence, it is reasonable to propose that the phytotoxicity displayed, *in vitro*, by water-extracts of *Calluna* peat may also be operative under field conditions.

Evidence to date suggests that the phytotoxin is a small, water-soluble

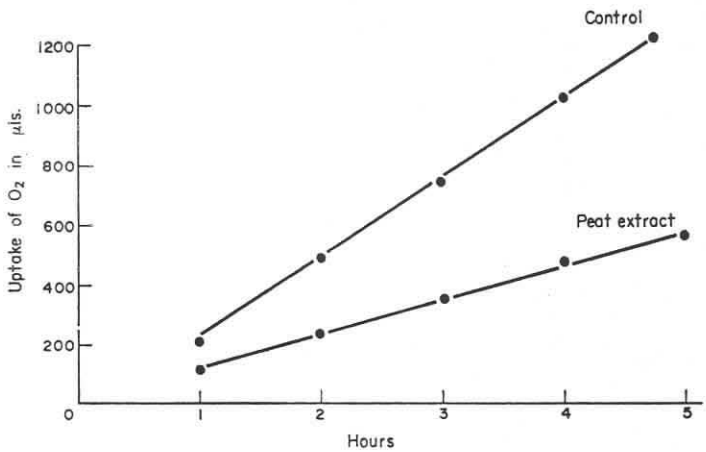

Figure 1. Uptake of oxygen in µlitres by tomato roots in the presence of water-extracts of *Calluna* peat.

molecule which is easily degraded even at the pH at which it normally occurs. It is derived from the decomposition of the *Calluna* litter, for incubation of freshly fallen leaf material gives rise to a phytotoxin in all respects identical to that present in water-extracts of naturally occurring peat. One last feature of the phytotoxic phenomenon which is of interest in relation to its role in the

field is that the level of detectable activity fluctuates markedly with season (see Fig. 2). This variation suggests that it is of biological origin, being produced in easily detectable amounts only when the summer rise in soil temperature stimulates the microflora into activity. This effect of temperature has been

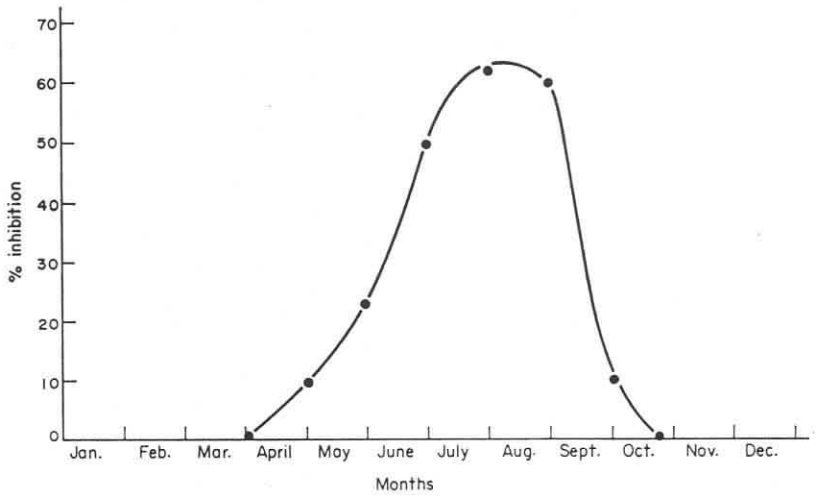

Figure 2. Seasonal variation in phytotoxicity of water-extracts of *Calluna* peat—percentage inhibition of oxygen uptake by tomato roots.

verified in the laboratory, and it is feasible to suppose that under environmental conditions suitable for the germination of seedlings, there is also a steadily increasing level of phytotoxicity. The role of this factor, therefore, in relation to the firing of heathland sites could well be important in terms of decreasing competition pressures during the re-establishment phase of *Calluna*, an effect enhanced later by the presence of a fungitoxic compound in the raw humus derived apparently from living *Calluna* plants.

Relation of the fungitoxic component to heathland ecology

It has been clearly established by Handley (1963) that water-extracts of peat from beneath healthy plants of *Calluna* do contain a substance inhibitory to the growth of certain mycorrhizal fungi in culture; moreover, this factor is barely detectable in peat samples under poorly developed plants, e.g. subject to shading. This evidence, together with the fact that the fungitoxin cannot be detected during the decomposition of *Calluna* litter, strongly suggests that the source of the mycorrhizal inhibitor is the roots of living *Calluna*, possibly as a secretion from the fine roots. It is true that evidence for the secretion of organic materials from the roots of a mature living plant is notable for its scarcity. However, there is one significant difference between most of the

plant species examined in this way and *Calluna*, namely that the latter has a very definite form of endotrophic mycorrhiza. This means that the fungitoxin need not be a normal metabolite of the *Calluna* itself, but rather the result of a combined fungus/host interaction.

It has already been shown (Condon & Kuc 1959; Martin 1958) that exposure of plant material to culture filtrates of specific fungi, or infection by the organism itself, can cause the host tissue to produce organic chemicals at levels far in excess of those normally present. It is reasonable to suggest, therefore, that the presence of the endophyte within the fine roots of *Calluna* could be associated with a similar effect. Indeed it is possible that the balance between host and endophyte in *Calluna* involves a chemical control system of the type found in orchids (Gaumann & Kern 1959). If this is so, then the secretion of a fungitoxin by the roots of *Calluna* becomes a feasible hypothesis, and in this context the findings of Stalder & Schutz (1957) working with *Erica gracilis* are of some interest. They found that when mycorrhizal associations of this species were eradicated by the application of nitrogenous fertilizers, then the roots succumbed to a heavy infection by a species of *Olpidium*. Plants with normal endotrophic mycorrhizas growing in the same soil showed no disease symptoms, and *Ericaceae* from natural habitats were never found to be infected by this pathogen. From their experiments they deduced that the mycorrhizal associate of *E. gracilis* exerted an antagonistic effect on *Olpidium*. A similar influence arising from the roots of *Calluna* could well explain the failure of certain ectotrophic mycorrhizal fungi to form associations with tree roots intertwined with those of heather.

Such a suggestion is not without parallel, for Munro (1966) has found that a dominant grass from the savanna in Southern Rhodesia secretes a toxin from its roots which differentially suppresses nitrifying bacteria. The secretion of this bacteriocidal material, when viewed in relation to the lowered competitive ability of *Hyparrhenia filipendula* in the presence of inorganic nitrogen, endows this species with a selective advantage for maintaining a dominant position in the grassland. A fungicidal secretion from the roots of *Calluna* could have a similarly selective effect, in that competing tree species would be prevented from reaching sufficient maturity to shade out the *Calluna*. Experimental evidence of the secretion of a fungicidal compound by the roots of *Calluna* has yet to be obtained, but circumstantial evidence strongly supports its existence.

Discussion

It is clear that on certain heathlands in this country dominated by *Calluna vulgaris*, the floristic stability of the community is, in part, maintained by compounds in the raw humus toxic to other plant species. One of these compo-

nents exerts a strong phytotoxic effect by inhibiting the growth of roots, while the other factor suppresses the formation of ectotrophic mycorrhizas on the roots of competing tree species.

The operation of these factors can best be envisaged by considering what happens when a section of heathland is cleared of living *Calluna* by fire, for at this point in time both groups of toxic factors will be reduced to a low level. It is in this situation, as was found by McPherson & Muller (1969) in considering the vegetation of the Chaparral, that plant species incapable of competing with the dominant flora on allelopathic grounds make an appearance. On heathland areas, tree seedlings, e.g. birch, as well as some herbaceous plants, such as *Holcus mollis*, become established, but after 4–5 years the flora is usually again Ericaceous with only a few stunted birches providing evidence of their former invasion. It is a reasonable hypothesis to suggest that this decline of competing species is due to the build-up of toxic factors in the raw humus. These toxic metabolites, localized in the raw humus layer, are well placed for their proposed role in suppressing root growth—an effect enhanced by the fact that, even if these toxins are unable to kill potential invaders, they may render them more susceptible to the ravages of other factors, for example, grazing. Support for such an idea comes from field observations that many unmanaged heaths are, since the decline of the rabbit, being colonised by woody species.

However, the very fact that birch may ultimately become established is of interest, for it implies a declining fungitoxic activity on the part of *Calluna*. It has been shown by Handley (1963) that any factor, e.g. shading, which adversely affects the heather leads to a lower level of fungitoxic activity in the raw humus beneath the plant. It is, therefore, clearly pertinent to ask whether the diminishing fungitoxic activity observed on unmanaged heaths is not due to the fact that the *Calluna* is approaching senescence, and further whether this senescence is not a manifestation of autotoxicity. For it has been shown on a number of occasions (cf. Miller & Miles 1970) that the ability of *Calluna* to regenerate after firing or cutting declines with age. One possible explanation of this phenomenon could be that the roots of *Calluna* are, to some extent, susceptible to the toxic metabolites building up in the raw humus, and that exposure to these factors over a number of years has a debilitating effect on the whole plant. Support for such an hypothesis is provided by the work of Muller (1966), who showed that an allelopathic influence from *Salvia leucophylla*, which restricted the growth of annual herbs, also caused a deterioration of the shrub itself. Whether or not auto-intoxication in this form exerts a real influence on the ecology of *Calluna* heaths remains to be established, but the fact that two distinct types of inhibitory material can be obtained in some quantity from heathland soils clearly indicates that such an hypothesis may not be without foundation.

The overall conclusion which may be drawn from our studies to date is that the presence of toxic compounds in heathland soils, whether providing stability to the community through antagonism to competing species or eventually opening the way to succession through auto-intoxication, is an ecological factor of almost unrealized potential. Obviously, there are many details of the general hypothesis which require critical examination before the role of soil toxicity in the ecology of heathlands can be assessed with authority, but the evidence is accumulating. If it can be shown that chemical antagonism between species is involved in maintaining the stability of *Calluna* heaths, then it is obviously pertinent to speculate upon the question of just how widespread this type of phenomenon may be. Certainly Muller (1966) has demonstrated one extremely clear-cut example of where the ecology of an area is dominated by the influence of toxic metabolites. Surely it is unreasonable to suppose that this is just an isolated occurrence. More likely his results form the tip of an uncharted 'ice-berg', the true dimensions of which will become apparent only when more plant associations have been examined along similar lines.

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Summary

The exposed nature of heathlands, together with the low mineral status of the soil and the susceptibility of the vegetation to burning, are all factors instrumental in ensuring the dominance of *Calluna vulgaris* over certain areas. Nevertheless, these factors may be subsidiary in importance to an allelopathic effect from the shrub itself. Thus, after the vegetation of some lowland heaths has been fired, trees, such as birch, can rapidly become established, only to fail some 4–5 years later. The apparent reason for this growth failure is the presence of phyto- and fungitoxic compounds in the raw humus; so that not only is growth of the birch roots restricted, but also the formation of efficient mycorrhizal associations.

Experiments have shown that a water-soluble factor is present in raw humus from beneath living *Calluna* which can inhibit root growth of a number of plant species, possibly through interfering with the respiration of the root tissue. Such a compound, absorbed on to the organic matrix of the peat, could well form a formidable barrier to the establishment of seedlings; an in-

hibition reinforced, in certain instances, by fungitoxic components. The chemical characteristics of the phytotoxic compound as examined in vitro support the view that it is ecologically significant in the role suggested. It is further speculated that this compound may be operative in dictating the growth pattern of *Calluna* itself, so that the eventual senescence of an individual bush may well be due, in part, to autotoxicity; the changing competitive ability of *Calluna* with observed growth phase, may also be correlated with the production of toxic metabolites.

References

BONNER J. (1950) The role of toxic substances in the interaction of higher plants. *Bot. Rev.* 16, 51–65.

BRAATHE P. (1950) Granas veksthemning pa lyngmark. *Tidsskr. Skogbr.* 58, 42–5.

BRIAN P.W., HEMMING H.G. & MCGOWAN J.C. (1945) Origin of a toxicity to Mycorrhiza in Wareham Heath soil. *Nature, Lond.* 155, 637–8.

CONDON P. & KUC J. (1959) A biochemical mechanism for the resistance of carrot root tissue to attack by *Ceratostomella fimbriata*. *Phytopathology* 49, 536.

GAUMANN E. & KERN H. (1959) Über chemische Abwehrreaktionen bei Orchideen. *Phytopath. Z.* 36, 1–26.

HANDLEY W.R.C. (1963) *Mycorrhizal associations and Calluna heathland afforestation.* Bull. For. Commn, Lond., No. 36.

HOAGLAND D.R. & ARNON D.I. (1938) *The water-culture method of growing plants without soil.* Circ. Calif. agric. Exp. Stn. 347.

LAING E.V. (1932) *Studies on tree roots.* Bull. For. Commn, Lond. No. 13.

MARTIN P. (1958) Einfluss der Kulturfiltrate von Mikroorganismen auf die Abgabe van Scopoletin aus den Keimwurzeln des Hafers (*Avena sativa* L). *Arch. Mikrobiol.* 29, 154–68.

MCPHERSON J.K. & MULLER C.H. (1969) Allelopathic effects of *Adenostoma fasciculatum*, 'Chamise', in the California Chaparral. *Ecol. Monogr.*, 39, 177–98.

MILLER G.R. & MILES J. (1970) Regeneration of heather (*Calluna vulgaris*) at different ages and seasons in north-east Scotland. *J. Appl. Ecol.* 7, 51–61.

MULLER C.H. (1966) The role of chemical inhibition (allelopathy) in vegetational composition. *Bull. Torrey bot. Club* 93, 332–51.

MULLER P.E. (1903) Über das Verhältnis der Bergkiefer zur Fichte im den Jutlandischen Heidekulturen. *Naturw. Z. Land- u. Forstw.* 1, 289–306.

MUNRO P.E. (1966) Inhibition of nitrifiers by grass root extracts. *J. appl. Ecol.* 3, 231–8.

RAYNER M.C. & NEILSON-JONES W. (1944) *Problems in tree nutrition.* Faber and Faber.

STALDER L. & SCHUTZ F. (1957) Untersuchungen über die kausalen Zusammenhange des Erikawurzelsterbens. *Phytopath. Z.* 30, 117–48.

TORREY J.G. (1959) A chemical inhibitor of auxin-induced lateral root initiation of *Pisum*. *Physiologia* Pl. 12, 873–87.

WOODS F.W. (1960) Biological antagonisms due to phytotoxic root exudates. *Bot. Rev.* 26, 546–69.

YEATMAN C.W. (1955) *Tree root development on upland heaths.* Bull. For. Commn, Lond. No. 21.